PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial
AUTHORS	Wang, Haibo; Ji, Ping; Zhao, Xing-Shan; Xu, Haiyan; Yan, Xiao-Yan; Yang, Qin; Yao, Chen; Gao, R; Wu, Yangfeng; Qiao, Shu-Bin

VERSION 1 - REVIEW

REVIEWER	Ioanna Kosmidou, MD PhD Columbia University/New York Presbyterian Hospital and Cardiovascular Research Foundation New York, NY, USA
REVIEW RETURNED	03-Apr-2017

GENERAL COMMENTS	The authors present a study protocol designed to address the safety and efficacy of rhTNK-tPA compared to alteplase in patients with STEMI. The study design follows acceptable standards for assessment of clinically pertinent cardiovascular endpoints and the rationale for the study is clear. Major comments: 1. It is not clear why rhTNK-tPA is compared to alteplase rather than the similar tenecteplase; if this latter product is not approved in China, this should be clearly stated or otherwise the choice of alteplase should be justified. 2. The authors indicate IRA patency by non invasive means as a secondary endpoint, however tit is not clear why the specified indirect indicators of IRA patency are based on; specific references to these indicators should be included 3. The authors should specify if the composite primary endpoint will be hierarchical 4. The authors should discuss whether Kaplan Meier curves will be used for assessment of MACCE at 30 days, in addition to MV regression. Further, the authors should clearly indicate whether the primary endpoint analysis will be based on the ITT vs per protocol population primarily Minor comments 1. MACCE should be clearly defined in the abstract. 2. There is no indication of an institutional approval process in each participating hospital, please clarify further. Also, please indicate whether investigators and the study Sponsor will have (or not) access to the data until the study completion. 3. Please move the discussion regarding the function of the DSMB to a more relevant section (it is currently under statistical considerations, it should be ideally moved either in the endpoint section or under a separate paragraph)

	4. Several unclear sentences should be rewritten; for example, on page 14 (par 1), it is stated that "... the efficacy and safety of Recomlyse can be assumed to be optional..."

REVIEWER	Wendy Ziai, MD Johns Hopkins University School of Medicine USA
REVIEW RETURNED	10-May-2017

GENERAL COMMENTS	A protocol is presented for a randomized controlled trial of rhTNK-tPA vs rt-PA in patients with STEMI in China at 150 sites who are unable to undergo PCI within 90 minutes. The study is planned as a non-inferiority analysis with primary endpoint of MACCEs within 30 days of fibrinolytic therapy. However, the objective appears more in line with improving “timely reperfusion therapy and hence treatment outcomes”. Therefore if the trial is positive, on what basis is it expected that tenectoplase would be chosen over alteplase? Given the results of the ASSENT-2 trial, what is the rationale for pursuing the current clinical trial? The protocol indicates that patients may be given rescue PCI as soon as possible if fibrinolysis fails. What percentage of patients are expected to undergo rescue PCI and how will use of rescue PCI be accounted for in the outcomes analysis? Of the secondary study endpoints, items (c)-(f) appear to be a repetition of the primary study endpoints. How are these different? The monitoring protocol suggests that the DSMB will review major bleeding events and safety data monthly. Will there be a planned interim analysis and at what point? Are safety thresholds determined for stopping the trial early? What is the composition of the CEC? How were the sites chosen for this trial? Are the sites considered settings where PCI is not immediately available? What proportion of patients are currently treated with fibrinolytics instead of PCI at these sites? The final sentence of the discussion appears to overstate the potential results of this study.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Ioanna Kosmidou, MD PhD

Columbia University/New York Presbyterian Hospital and

Cardiovascular Research Foundation, New York, NY, USA

Please state any competing interests or state ‘None declared’: None declared

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Please leave your comments for the authors below

The authors present a study protocol designed to address the safety and efficacy of rhTNK-tPA compared to alteplase in patients with STEMI. The study design follows acceptable standards for assessment of clinically pertinent cardiovascular endpoints and the rationale for the study is clear.

Major comments:

1. It is not clear why rhTNK-tPA is compared to alteplase rather than the similar tenecteplase; if this latter product is not approved in China, this should be clearly stated or otherwise the choice of

alteplase should be justified.

Thanks for reviewer's insightful suggestions. As reviewer speculates, tenecteplase is not approved in China. We have added the related information in the Introduction section as following:
"tPA has been approved by China FDA, but Tenecteplase has not yet entered the Chinese market."

2. The authors indicate IRA patency by non invasive means as a secondary endpoint, however tit is not clear why the specified indirect indicators of IRA patency are based on; specific references to these indicators should be included

The gold standard for the diagnosis of failed reperfusion is a combination of coronary angiography and myocardial contrast imaging. However, some reliable non-invasive marker has been incorporated into clinical routine use for convenience, including resolution of chest pain, ST segment resolution, and biochemical diagnosis of failed thrombolysis. The related references have been cited in our revised manuscript:
Davies CH, Ormerod OJ. Lancet. 1998; 351(9110):1191-6.
Chinese Journal of Cardiology Editorial Board. Reference scheme for treatment of fibrinolytic therapy for acute myocardial infarction. Chinese Journal of Cardiology 1996; 24(5):328-29. (In Chinese)

3. The authors should specify if the composite primary endpoint will be hierarchical.

Our primary endpoint is MACCEs without hierarchical structure. But we would like to hear your advices.

4. The authors should discuss whether Kaplan Meier curves will be used for assessment of MACCE at 30 days, in addition to MV regression. Further, the authors should clearly indicate whether the primary endpoint analysis will be based on the ITT vs per protocol population primarily.

Kaplan Meier curves will be used to depict the occurrence of MACEE within 30 days of fibrinolytic therapy, and it has been shown in our statistical analysis methods as following:
"Survival curves of MACCEs within 30 days of fibrinolytic therapy were estimated by Kaplan-Meier method and compared by log-rank test."
We have described the analysis population definition in statistical analysis methods as following:
"Both intention-to-treat (ITT) and per-protocol analyses will be done for the primary analyses as is recommended for non-inferiority studies, but principally with reference to per-protocol analysis."

Minor comments
1. MACCE should be clearly defined in the abstract.

We have supplemented the definition of MACCE in abstract as following:
"MACCEs were defined as comprising all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), percutaneous coronary interventions (PCI) due to thrombolysis failure and PCI due to reocclusion."

2. There is no indication of an institutional approval process in each participating hospital, please clarify further. Also, please indicate whether investigators and the study Sponsor will have (or not) access to the data until the study completion.

We have clarified this question in Ethical approval section as following:

“The protocol and informed consent form have been reviewed and approved by all participating hospitals”
Privilege of data access is indicated the in the Competing interests section as following:
“Principal investigator has full access to the final trial data set, but the sponsor doesn’t have access to the data.”

3. Please move the discussion regarding the function of the DSMB to a more relevant section (it is currently under statistical considerations, it should be ideally moved either in the endpoint section or under a separate paragraph)

As suggested, the description on the function of the DSMB has been setup as a separate paragraph named as “Safety data monitoring” following Study endpoints section.

4. Several unclear sentences should be rewritten; for example, on page 14 (par 1), it is stated that "... the efficacy and safety of Recomlyse can be assumed to be optional..."

Thanks for reviewer’s kind reminding. It has been revised as following:
“the efficacy and safety of rhTNK-tPA (Recomlyse®) can be assumed to be ideal”

Reviewer: 2
Wendy Ziai, MD
Johns Hopkins University School of Medicine, USA
Please state any competing interests or state ‘None declared’: None declared
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Please leave your comments for the authors below
A protocol is presented for a randomized controlled trial of rhTNK-tPA vs rt-PA in patients with STEMI in China at 150 sites who are unable to undergo PCI within 90 minutes.
The study is planned as a non-inferiority analysis with primary endpoint of MACCEs within 30 days of fibrinolytic therapy. However, the objective appears more in line with improving “timely reperfusion therapy and hence treatment outcomes”. Therefore if the trial is positive, on what basis is it expected that tenectoplase would be chosen over alteplase?

Thanks reviewer for his constructive suggestions on the current protocol. Indeed, timely reperfusion is the key to reduce mortality and morbidity of STEMI patients. However, it is not the basis for conducting the current study. rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Whereas rhTNK-tPA has significant advantages compared with rt-PA, including ease administration as a bolus, longer half-life and better fibrin specificity.
It is hard to prove the superiority of therapeutic effect of rhTNK-tPA compared with rt-PA, especially in clinical trials in which rigorous management was conducted to keep high quality of study. However, for the treatment of acute disease such as STEMI, convenience is one of the important factors to be considered in first-aid. Inconvenience may result in indirect therapeutic effect in common clinical practices. Therefore, we think convenience in first-aid is one important reason for considering rhTNK-tPA in clinical practices.

Given the results of the ASSENT-2 trial, what is the rationale for pursuing the current clinical trial?

ASSENT-2 trial, in which 16949 participants from more than 1,000 hospitals in 29 countries were randomized, showed that single-bolus tenecteplase and front-loaded altiplase had equivalent effect on 30-day mortality.
Tenecteplase (TNKaseTM, Genentech Inc.), has been approved by the US Food and Drug Administration (FDA) in 2000, but Tenecteplase has not yet entered the Chinese market. The corresponding information has been shown in our background section.
Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA, Recomlyse®, Guangzhou Recomgen Biotech Co., Ltd.) with the same amino acid sequence with tenecteplase, has been developed by Guangzhou Recomgen Biotech Co.,Ltd., China. The difference in therapeutic effects between rhTNK-tPA and Tenecteplase has not been studied in Chinese population although similar study was conducted in other countries. Moreover, it is the requirement of China FDA to further validate its' efficacy and safety in clinical practices. In ASSENT-2 trial, the primary endpoint was all-cause mortality at 30 days, whereas in the current study, the primary endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. The primary endpoint in the current study can more comprehensively reflect the efficacy of fibrinolytic therapy. Therefore, it is necessary to conduct the current study to validate efficacy and safety of rhTNK-tPA among Chinese population.

The protocol indicates that patients may be given rescue PCI as soon as possible if fibrinolysis fails. What percentage of patients are expected to undergo rescue PCI and how will use of rescue PCI be accounted for in the outcomes analysis?

It is estimated that 12% participants would undergo rescue PCI due to fibrinolysis failure. Rescue PCI has been included in our primary endpoint to account its' impact. The primary study endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion.

Of the secondary study endpoints, items (c)-(f) appear to be a repetition of the primary study endpoints. How are these different?

The primary endpoint is a composite endpoint in the current study, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. In addition to the composite endpoint, we also hope determining the efficacy and safety on each of these single component of the primary endpoint. Moreover, it is a common practice in clinical research.

The monitoring protocol suggests that the DSMB will review major bleeding events and safety data monthly. Will there be a planned interim analysis and at what point? Are safety thresholds determined for stopping the trial early?

Thanks reviewer for his important suggestion on this topic. As this trial is a non-inferiority study, an interim analysis is not considered and statistical early stopping criteria will not be applied. The DSMB is not going to meet on monthly intervals. The safety data analyses should be done after 10%, 25% and 50% patients completing the study procedure respectively, and DSMB may recommend stopping the study anytime based on safety concerns.
We have added related information in "Safety data monitoring" section.

What is the composition of the CEC?

We have supplemented the detailed information about CEC composition, appointment and management in the manuscript as following:
"CEC is composed of four cardiovascular specialists and one neurological expert who are recommended and appointed by Peking University Clinical Research Institute. CEC which is blinded to treatment assignment independently evaluates the primary outcome and safety indicators. Additional two secretaries help collect supporting information about endpoints assessment, organize the communication of evaluation committee and summarize the final results."

How were the sites chosen for this trial? Are the sites considered settings where PCI is not immediately available? What proportion of patients are currently treated with fibrinolytics instead of PCI at these sites?

About 150 sites will be chosen for the current study as it is estimated that STEMI patients in these hospitals are unable to undergo PCI within 90 minutes. It is estimated that about 80% participating hospitals have no facilities to provide emergency PCI.

The final sentence of the discussion appears to overstate the potential results of this study.

We agree with reviewer's suggestion and have revised the sentence as following:
"Not surprisingly, once the efficacy and safety of rhTNK-tPA is confirmed in the study, its application in China would help improve the treatment of STEMI patients based on its potential advantages including ease of bolus administration, longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor."

VERSION 2 – REVIEW

REVIEWER	Ioanna Kosmidou, MD PhD Columbia University and Cardiovascular Research Foundation, USA
REVIEW RETURNED	22-Jun-2017

GENERAL COMMENTS	Most comments have been addressed and the manuscript improved. I suspect that the utilization of a non-hierarchical composite endpoint will be concerning and suggest that the authors consider statistical analyses based on hierarchical pairwise comparisons with the Finkelstein-Schoenfeld methodology or the Win-ratio statistic by Pocock or at least explain the rationale behind utilization of a non-hierarchical composite.

REVIEWER	Wendy Ziai, MD, MPH Johns Hopkins Medical Institutions
REVIEW RETURNED	27-Jun-2017

GENERAL COMMENTS	The manuscript is significantly improved and all reviewer queries have been answered. Minor revision for English grammar required. Please put statistical plan into the future tense; ie. "Survival curves of MACCEs within 30 days of fibrinolytic therapy were estimated by Kaplan-Meier method and compared by log-rank test" - change to "will be".

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Ioanna Kosmidou, MD PhD
Columbia University and Cardiovascular Research Foundation, USA
Please state any competing interests or state 'None declared': None declared
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Please leave your comments for the authors below
Most comments have been addressed and the manuscript improved. I suspect that the utilization of a non-hierarchical composite endpoint will be concerning and suggest that the authors consider statistical analyses based on hierarchical pairwise comparisons with the Finkelstein-Schoenfeld methodology or the Win-ratio statistic by Pocock or at least explain the rationale behind utilization of a non-hierarchical composite.

Thanks for reviewer's further suggestions on this point. But as we know, a non-hierarchical composite endpoint has been used in similar studies:
Sinnaeve PR, Alexander JH, Bogaerts K, Belmans A, Wallentin L, Armstrong P, Adgey JA, Tendera M, Diaz R, Soares-Piegas L, Vahanian A, Granger CB, Van De Werf FJ. Efficacy of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: one-year follow-up results of the Assessment of the Safety of a New Thrombolytic-3 (ASSENT-3) randomized trial in acute myocardial infarction. Am Heart J. 2004 Jun;147(6):993-8.
Wallentin L, Goldstein P, Armstrong PW, Granger CB, Adgey AA, Arntz HR, Bogaerts K, Danays T, Lindahl B, Mäkijärvi M, Verheugt F, Van de Werf F. Efficacy and safety of tenecteplase in combination with the low-molecular-weight heparin enoxaparin or unfractionated heparin in the prehospital setting: the Assessment of the Safety and Efficacy of a New Thrombolytic Regimen (ASSENT)-3 PLUS randomized trial in acute myocardial infarction. Circulation. 2003 Jul 15;108(2):135-42.
Pastori D, Carnevale R, Nocella C, Novo M, Santulli M, Cammisotto V, Menichelli D, Pignatelli P, Violi F. Gut-Derived Serum Lipopolysaccharide is Associated With Enhanced Risk of Major Adverse Cardiovascular Events in Atrial Fibrillation: Effect of Adherence to Mediterranean Diet. J Am Heart Assoc. 2017 Jun 5;6(6).
We can't determine utilization of hierarchical pairwise comparisons with the Finkelstein-Schoenfeld methodology or the Win-ratio statistic by Pocock at present, but we can consider the problem in the future when the study is completed.

Reviewer: 2
Wendy Ziai, MD, MPH
Johns Hopkins Medical Institutions
Please state any competing interests or state 'None declared': None Declared
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Please leave your comments for the authors below
The manuscript is significantly improved and all reviewer queries have been answered. Minor revision for English grammar required. Please put statistical plan into the future tense; ie. "Survival curves of MACCEs within 30 days of fibrinolytic therapy were estimated by Kaplan-Meier method and compared by log-rank test" - change to "will be".

The future tense is used in statistical analysis section as suggested.